5.0 THE ASBESTOS LITERATURE

This is a description of the common types of studies in the asbestos literature and an overview of the sources of potential uncertainty typically associated with each. Such limitations must be considered when drawing conclusions from these studies and, more importantly, when comparing inferences across studies. Throughout this document, we have endeavored to identify the major sources of uncertainty in the studies we examined and have endeavored to account for such uncertainties during our evaluation and interpretation of study results.

The types of studies available for examining relationships between risk and asbestos exposure include human epidemiology studies, human pathology studies, a broad variety of animal studies, and a broad variety of in-vitro studies in both tissue cultures and cell-free systems. To properly compare and contrast the results from such studies:

- the method(s) employed for asbestos characterization in each study need to be reconciled;
- the procedures employed for evaluating study end points need to be compared and contrasted;
- the relationship between the route of exposure employed in each type of study and the exposure route of interest (i.e. human inhalation) needs to be examined; and
- other major, study-specific sources of uncertainty need to identified and addressed.

Among study conditions and procedures that must be considered before evaluating study conclusions, it is particularly important to address the analytical methodologies employed to characterize the nature of exposures (or doses) in each study and such considerations are common to virtually all of the various types of studies of interest.

As indicated in Section 4.3, the only instrument capable of completely delineating asbestos structure size distributions is TEM (or TEM combined with other techniques). Thus, for example, conclusions regarding variations in biological effects due to differences in such things as fiber size must be viewed with caution when fiber sizes are characterized using only PCM, SEM, or other, cruder analytical techniques. Similarly, the ability to adequately determine fiber mineralogy (fiber type), particularly of what may be minor constituents of various dusts or bulk materials, also depends strongly on the instrumentation employed for analysis as well as the strategy for sampling and for conducting the actual structure count. All of these factors must be considered.

Not only does the specific instrumentation (analytical technique) that is employed in an asbestos measurement affect the outcome of that measurement, but the particular method employed to guide the measurement affects the outcome. As previously indicated (Section 4.3), details concerning the definition of structures to be included in a count, the strategy for counting, and the minimum number of specific types of structures to be included in a count (all features that vary across analytical methods) affect the precision with which fiber concentrations (particularly of longer and thinner fibers) are delineated. It is not uncommon, for example, that asbestos concentrations measured in the same sample may vary by several orders of magnitude, due simply to a difference in the analytical method employed for the analysis (even when the same analytical technique is employed, see Section 4.3).

Other important sources of uncertainty tend to be study-type specific and are thus addressed separately below.

5.1 HUMAN EPIDEMIOLOGY STUDIES

A good overview of the kinds of limitations that contribute to uncertainty in the available epidemiology studies was presented in the Health Effects Assessment Update (U.S. EPA 1986). As described in Appendix A of this document, while evaluating dose-response factors derived from the human epidemiology studies, we tried to address most of the major sources of uncertainty commonly associated with such studies, which are described briefly below.

Epidemiology studies, which track the incidence of disease (or mortality) within a defined group (cohort) sharing comparable exposures, have been performed on cohorts of workers exposed to asbestos and other mineral fibers in a variety of occupational and environmental settings. Among these, studies that include quantification of exposures are particularly useful for evaluating dose/response relationships and deriving risk factors.

Generally, the most severe limitations in an epidemiology study involve the exposure data. Both estimates of the level of exposure and determination of the character of exposure are affected by such limitations. Regarding the character of exposure, because exposure measurements from most of the available quantitative epidemiology studies are based on midget impinger measurements or PCM measurements, detailed characterization of the size distribution or the mineral type of fibrous structures (particularly of minor constituents) that contributed to exposure in such studies is generally lacking (Section 4.3). This is particularly important because of the evidence that neither midget impinger or PCM are capable of providing measurements that remain proportional (across study environments) to the biologically-relevant characteristics of an asbestos dust (see Section 7.5 and Appendix B). This limits the

ability both to compare results across the existing studies and to extrapolate such results to new environments for which risks need to be estimated. At the same time, effects on the ability to observe dose-response trends within a single study are not typically impaired.

Samples collected prior to the mid-1960s were often analyzed by measuring total dust in units of millions of particles per cubic foot (MPCF) using impingers or thermal precipitators. A description of the relative strengths and weaknesses of these techniques is provided in Section 4.3. The fibrous portion of the dust was not monitored. Impinger measurements are sometimes related to fiber counts (based on PCM) using side-by-side measurements of total dust and fiber counts collected during a relatively brief period of time (e.g., McDonald et al, 1980b, Dement et al, 1983a). However, the correlation between fiber counts and total dust is sometimes poor within a plant and generally poor between plants (see, for example, U.S. EPA 1986). Thus, conversions based on limited sets of paired measurements are of questionable validity. In some studies (e.g., McDonald et al, 1983b) the only available measurements are impinger measurements in MPCF and these have been related to f/ml by PCM using conversion factors derived in other plants, which raises further questions concerning validity.

Even conversion to PCM may not be adequate for assessing risk in a manner that allows extrapolation across exposure environments or studies. As indicated in Section 7.5, comparing dose-response factors derived in different exposure environments (or extrapolating to new environments to predict risk) requires that asbestos measurements reflect the characteristics of asbestos structures (size, shape, mineralogy) that determine biological activity. If surrogate measures are employed (e.g. measures of asbestos structures displaying characteristics other than those that determine biological activity), there is no guarantee that concentrations of such surrogate measures and the true biologically active structures will remain proportional from one environment to the next. As a consequence, the relationship between exposure (measured by surrogate) and risk may not remain constant from one environment to the next. Importantly, several studies suggest that PCM may, at best, be no more than a surrogate measure (see, for example, Berman et al. 1995). Moreover, the technique was adapted to asbestos in an ad hoc fashion with only limited thought given to biological relevance (Walton 1982).

Use of surrogate measures of asbestos exposure may be less of a problem within a single exposure environment (where airborne asbestos structures likely have been generated in a similar manner from similar source material). Thus, surrogate measures of asbestos exposure may remain approximately proportional to the true biologically active structures, which suggests why monotonically increasing dose-response relationships have likely been observed with PCM-measured concentrations *in single exposure environments.* In different exposure environments, however, airborne

asbestos structures likely are generated in different processes from different source material. There is thus little reason to expect surrogate measures of exposure to remain proportional across such environments.

None of the published epidemiology studies incorporate TEM measurements of asbestos and such measurements are not widely available in occupational settings (Section 6.2.4). However, TEM is the method currently used (and recommended) to assess exposure in environmental settings (due both to questions concerning biological relevance (Section 7.5) and to problems with measuring environmental asbestos concentrations by PCM (Section 4.3 and Appendix B).

In some cases, the limited exposure characterization presented in specific epidemiology studies can be augmented by pairing such studies with published TEM characterizations of dusts from the same or similar exposure settings, to the extent the appropriate supplemental studies are available. In fact, this is the procedure adopted in this document to adjust the existing risk factors to exposure indices that are thought to better relate to biological activity (Sections 6.2.4 and 6.3.3). Such an approach is limited, however, to the extent that the published asbestos characterizations actually represent exposure conditions in the corresponding epidemiology studies. To the extent reasonable, the limitations of this approach have been addressed in this study by assigning and incorporating additional factors into the calculation of the confidence intervals associated with the adjusted potency factors (Sections 6.2.4 and 6.3.3).

Regarding levels of exposure in the epidemiology studies, in most cases, air measurements were collected only at limited points in time and measurements may be entirely lacking from the earliest time periods, when exposures may have been heaviest. In such cases, exposures are typically estimated either by extrapolation from periods when measurements are available or by expert judgement based on personal accounts and records of changes in plant operations, industrial hygiene procedures, air standards, etc. Moreover, the majority of exposure measurements used in these studies are based on area (ambient) rather than personal samples. Typically, only a few areas of a plant have been sampled so that levels in other areas must be approximated using expert judgement by persons familiar with operations at the plant.

It is difficult to judge the degree that available asbestos concentration measurements are representative of actual exposures in the existing studies. In some cases, it seems likely that operations were shut down or otherwise modified in preparation for sampling. Likewise, in some operations there are brief episodes of very intense exposure and it is questionable whether such episodes are adequately represented in the available data.

Most of the asbestos measurements used in the published epidemiology studies were collected for insurance or compliance purposes. They were not intended to provide representative estimates of the direct level of exposure to workers. Some of the

published epidemiology studies lack any direct exposure data. For example, exposures were estimated for the cohort studied by Seidman (1984) based on conditions simulated many years later in a similar plant to the one from which Seidman studied the original cohort. In fact, the equipment in the plant from which Seidman obtained exposure estimates came originally from the plant where Seidman studied the workers; it was purchased and moved. Recently, an epidemiology study was also completed for a cohort working at that new plant (Levin et al. 1998).

In addition to problems with the actual analysis of asbestos concentrations, individual exposures are generally estimated in the existing epidemiology studies by relating ambient asbestos measurements to job descriptions and integrating the duration of exposure over the recorded time that each worker spent in each job category. However, sometimes there are no records of specific areas in which an employee worked, so that work areas must be assumed based on job title. Some types of workers (e.g., maintenance workers) may have spent time in many different areas of a plant so their exposure varies from what might otherwise be assumed.

Although the greatest problems with the data in existing epidemiology studies likely lies within the estimates of exposure, problems with disease-response data also exist. Mesothelioma is rare and this disease may have been under-reported as a cause of death in older studies. This is probably less of a problem in more recent studies, since the association of mesothelioma with asbestos exposure is now well known. In fact, the opposite tendency (over-reporting) may now be occurring because an asbestos worker with mesothelioma is probably eligible for compensation. Some studies have re-diagnosed causes of death from all of the available data (e.g., Selikoff et al, 1979); however, this creates the problem of lack of comparability to control populations (for which such re-diagnosis is not generally performed).

The choice of an appropriate control population is also an important consideration. Local cancer rates may differ substantially from regional or national rates and the choice of an appropriate control is not always clear. A related problem is the lack of smoking data in many of the studies. Because of the interrelation between smoking and asbestos in lung cancer, errors could occur in lung cancer risk estimates if the smoking patterns of the cohort are substantially different from those of the control population.

In some of the studies, a substantial portion of the population is lost to follow-up (e.g., Armstrong 1988), and this adds additional uncertainty to the analysis. Also, the effect of exposure may be inaccurately evaluated if the follow-up of the population is too brief. This may be a limitation, for example, of the Levin et al. (1998) study.

Another problem frequently associated with these studies is that available data are not reported in a form that is well-suited to risk assessment. The EPA lung cancer model,

for example, requires that exposure be estimated as cumulative exposure in f/ml·years excluding the most recent 10 years (Section 6.2); generally the data are not published in this form. The data are also frequently not available in a form that permits study of the shape of the lung cancer dose/response curve, so it is not possible to determine how well the EPA model describes the data. The form of the data for mesothelioma is generally even less appropriate for risk assessment. Ideally, what is needed is the incidence of mesothelioma subdivided according to exposure level, age at beginning of exposure, and duration of exposure (Section 6.3). Such data are almost never available in published studies and crude approximations must be made to account for this lack.

It is important to appreciate the type and magnitude of effect that each of these sources of uncertainties are likely to have on the distribution of potency estimates derived from the set of available studies for lung cancer and mesothelioma, respectively. Some of the above-described limitations likely introduce random errors that simply decrease the overall precision of a potency estimate. However, other types of limitations may cause systematic errors in particular studies, which potentially biases the potency estimate either high or low. Some of the limitations may only affect between-study differences and some may introduce a systematic bias between either industry types or fiber types. Examples of some of these types of variation are provided in Section 6.1.

We also note that, although individual estimates of potency factors from individual studies may be highly uncertain, by combining results across multiple studies while properly addressing such uncertainties, it may be possible to draw conclusions with greater precision than reasonable for any individual study. This is the essential advantage of the type of meta analysis discussed in Section 6.2.4 for lung cancer and 6.3.3 for mesothelioma.

5.2 HUMAN PATHOLOGY STUDIES

Human pathology studies provide a characterization of disease morphology and correlations between causes of death and the types of asbestos fibers retained in the lungs and other bodily tissues. These studies generally involve microscopic examination of tissue samples for indications of morphologic changes characteristic of disease and/or microscopic examination of digested tissue specimens to characterize the mineral fibers extracted from such tissue.

The results of human pathology studies need to be evaluated carefully by addressing effects that are attributable to:

- the way that tissue samples are fixed for preservation;

- the way that tissue samples are prepared for analysis (e.g. ashing, bleach digestion, digestion in alkali, or some combination);
- the choice of methods employed for characterization of asbestos; and
- the choice of locations within tissues from which samples are collected for analysis.

Because tissue samples obtained from deceased individuals are typically stored for long periods of time before they may be analyzed as part of a human pathology study, such samples are commonly fixed by treatment with chemical preservatives prior to storage. However, Law et al. (1991) studied the effects of two common fixatives (Karnovsky's fixative and formalin fixative) on asbestos fibers and concluded that such fixatives degrade and dissolve asbestos fibers (including both chrysotile and crocidolite) at measurable rates (Section 7.2.4). Therefore, particularly for samples that are stored "wet" (as opposed, for example, to storage in paraffin blocks), the concentrations and character of the tissue burden of asbestos may be altered during storage. Even for studies in which relative (as opposed) to absolute concentrations are being compared, alterations associated with preservation may limit the ability to make such comparisons, particularly among samples stored for widely disparate periods of time or stored using widely disparate procedures.

Fiber concentrations in tissue samples have also been shown to vary as a function of the method employed for preparing such samples for analysis. Historically, samples that are digested in bleach or alkali have tended to exhibit lower recovery of asbestos fibers than samples that are ashed (**REF**). However, more recent studies suggest that improving technique has narrowed these differences so that this is no longer a major consideration (**REF**). Thus, when comparing results across studies, due consideration needs to be given for the time frame during which such studies were conducted and the comparability/differences in the techniques employed for tissue sample preparation.

Once prepared, both the character and the concentration of the tissue burden measured in a tissue sample will also depend heavily on the particular analytical method employed to characterize asbestos and differences attributable to such techniques must be reconciled before measurements across samples or conclusions across studies can be reasonably compared. A more detailed description of the effects attributable to asbestos measurement was presented in the previous section on human epidemiology studies (Section 5.1) and the same issues obtain for human pathology studies. Unless measurements are made using comparable instrumentation with comparable methodology, comparisons across such measurements can be very misleading.

Perhaps the biggest limitation hindering the kinds of evaluation that can be conducted based on human pathology studies is that due to the strong dependence of asbestos concentrations on the specific location within a tissue from which a sample is obtained. Numerous authors have reported that asbestos is non-uniformly distributed in lung parenchyma and other tissues following exposure (see, for example: Davis et al. 1986a, Bignon et al. 1979, or Pooley 1982). The incidence of lesions and other pathological effects attributed to asbestos exposure correspondingly exhibit a non-uniform distribution.

For lung tissue samples (which tend to be among the primary interests in human pathology studies) the relationship between sample location and asbestos concentration is particularly important. To sample deep lung tissue reproducibly, it has been shown necessary to select a specific section of lung parenchyma from a defined portion of the bronchio-alveolar tree. Pinkerton et al. (1986) showed that the deposition of asbestos in the lungs is an inverse function both of the pathlength and the number of bifurcations between the trachea and the site. Thus, analyses of samples from different animals of the same species can only be compared meaningfully if the samples are collected from identical locations in the bronchio-alveolar tree. Similar, nonuniform depositional patterns have also been observed in humans (Raabe 1984). Futhermore, due to the complex branching and folding pattern of the lung, adjacent sections of lung parenchyma frequently represent disparate portions of the bronchio-alveolar tree (**REF**). Consequently, lung burdens derived even from adjacent samples of lung parenchyma can show broadly varying concentrations (dffering by orders of magnitude).

Unfortunately, however, tissue samples that are available for analysis in support of a human pathology study are typically “opportunistic” samples, which means that they were selected and stored for an entirely different purpose, usually with little or no thought given to the specific location from which they were collected (except in the most general way). It is therefore seldom possible to address the effects of sample location directly. Consequently, comparisons of tissue burden concentrations across samples from different individuals in a human pathology study are at best qualitative and may only be useful when averaged over large numbers of individuals and only when large differences in concentrations (several orders of magnitude) are being distinguished. Moreover, because the parts of a tissue that undergoes morphologic changes induced by asbestos typically corresponds to the parts of a tissue where asbestos burdens are the highest, even comparison of morphologic effects across tissue samples requires proper consideration of the effects of the locations from which such tissue samples were derived.

5.3 ANIMAL STUDIES

In animal studies, various species are exposed to measured doses of size-selected mineral fibers and the resultant biological responses are monitored. Animals may be

dosed either by inhalation, intratracheal installation, implantation, or injection (U.S. EPA 1986). Such studies are conducted for several purposes. As with human pathology studies, animal pathology studies are those in which the transport of asbestos structures is tracked through the various organs and tissues of the animal and the attendent cellular and molecular changes are characterized. In parallel with quantitative epidemiology studies, animal dose/response studies track the incidence of disease among a population that has been exposed in a controlled manner. One of the advantages of animal dose/response studies over epidemiology studies is that exposures are controlled and can be well characterized. The major disadvantage is that there are many uncertainties introduced when extrapolating the results of animal data to predict effects in humans. Therefore, attempts to adapt such things as animal derived dose-response factors to humans are not generally recommended.

As with human epidemiology and pathology studies, the validity of conclusions drawn from animal studies depends strongly on the techniques and methods used to characterize and quantify asbestos structures either in the delivered dose or in the tissues of the dosed animals (see Section 5.1). The ability to reconcile conclusions derived from many animal studies with the rest of the asbestos literature is limited because SEM was commonly employed to measure asbestos in animal studies but not other kinds of studies. Even many of those studies in which TEM was employed for asbestos analysis suffer from use of non-standard methods that cannot be easily reconciled with the more traditional TEM methods, particularly because such specialized methods are seldom adequately documented to allow comparison.

As with human pathology studies, the location of a tissue sample excised for analysis is a critical factor that also governs the quality of an animal pathology study (Section 5.2). However, one potential advantage frequently available in animal pathology studies over human studies is the ability to carefully identify and select the precise tissue samples to be analyzed. The extent that a particular animal pathology study exploits this capability can affect the overall utility of the study. Thus, such issues need to be addressed carefully when evaluating and comparing the results across animal pathology studies.

The route of exposure employed in a particular animal study is also important to consider. Each of the routes of exposure commonly employed in these studies (inhalation, intratracheal installation, and injection or implantation) delivers different size fractions of asbestos to a target tissue with varying efficiencies. For example, injection or implantation studies deliver 100% of all size categories of structures to the target tissue. However, the efficiency that each size category is delivered by inhalation is a function of the aerodynamic properties of the asbestos structures and the air flow characteristics of the lungs (Section 7.1). Thus, the relationship between dose and exposure depends upon the route of exposure employed. Importantly, because the ultimate goal is to understand the effects that inhaled fibers have on humans, differences between the character of the delivered dose in an animal study and the

character that such a dose would have, had it originally been inhaled, typically need to be addressed.

Regarding the measurement of health effects, many of the results in animal studies suffer from a lack of statistical significance because of small numbers of observed tumors. Consequently, trends cannot be established conclusively. Interestingly, several studies draw conclusions regarding the relative potency of different sample types when variations within the dose/response trend of a single sample type is larger than observed differences between types (**REFERENCE**).

For animal inhalation studies, meaningful comparison of the relative deposition of asbestos dusts across species is not direct. To extrapolate results across species, the detailed differences in the physiology of the respiratory tracts between the species need to be addressed (Sections 4.4 and 7.1). However, if measurements are available for both species, differences in physiology are addressed, and the manner in which tissue burdens are analyzed is considered, it may be possible to compare relative tissue doses (mass of asbestos per mass of tissue) across species.

5.4 IN-VITRO STUDIES

A broad range of in-vitro studies provide useful insight on the effects of asbestos. These include, for example, studies in cell-free systems (which have been used to evaluate such things as asbestos dissolution rates or the kinetics of free radical formation on the surface of asbestos fibers) and studies of the effects of asbestos on cultures of a broad variety of cell types and tissues.

As with other studies, the potential limitations and sources of uncertainty associated with in-vitro studies need to be considered when evaluating the validity of study results or comparing such results to those of other studies, particular studies of varying type. Also, as with other studies, among the primary sources of uncertainty that need to be addressed for in-vitro studies is the manner in which asbestos doses are characterized and quantified (Section 5.1). For in-vitro studies (as with animal studies dosed by routes other than inhalation), this also extends to the need to consider the relationship between the character of the asbestos dose applied in vitro and the character that a similar exposure might possess following inhalation exposure in vivo (Section 5.3). This can be particularly problematic for studies in tissue cultures because it is not clear how the application of a suspension of fibers (with known concentration) to a dish containing cultured cells can be related to doses that reach corresponding tissues following administration to whole animals.

In-vitro studies, of necessity, represent isolated components of living systems observed under conditions that may vary radically from those under which such components operate in vivo. Consequently, the behavior of such components may also vary

radically from the behavior of the same components in vivo. Therefore, additional study-specific considerations (concerning the design of a study and the conditions under which a study is conducted) also need to be addressed before evaluating the validity or relevance of the results from an in-vitro study to what might otherwise be observed in a whole animals. Examples of such considerations include:

– for cell-free systems:

  - whether conditions under which the study is conducted are sufficiently similar to conditions in vivo to expect that the observed effect is likely to occur in vivo; and

  - if the observed variables describing the nature or magnitude of the effect are also likely to reflect what may occur in vivo;

– for tissue cultures:

  - whether conditions under which the study is conducted are sufficiently similar to conditions in vivo to expect that the observed effect is likely to occur in vivo;

  - whether responses by specific tissues or cells in culture are likely to behave similarly in vivo where their behavior may be suppressed, enhanced, or modified in some other manner due to additional stimuli provided by responses of other tissues and cells that are components of a complete organism but that may be lacking in culture; and

  - whether the conditions required to establish and maintain a tissue culture for experimentation sufficiently alters the characteristics and behavior of the cells being studied to minimize the relevance of results from such a study to conditions in vivo.

Among the most important examples of the last consideration relates to the general need to create immortalized cells to maintain tissue cultures. Thus, questions must always be raised concerning whether the alterations required to create immortalized cells for culture (from what are normally mortal cells in vivo) also alter the nature of the responses being studied.

Note, many studies in the current literature also incorporate combined aspects of several of the four general study types described in this chapter. For these studies, a corresponding combination of the considerations described must therefore be addressed when evaluating such studies and comparing their results with inferences derived from the rest of the literature.